



Research Paper

# Differential SERM activation of the estrogen receptors (ER $\alpha$ and ER $\beta$ ) at AP-1 sites

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## Abstract

**Background:** The selective estrogen receptor modulators (SERMs) raloxifene and tamoxifen are triphenylethylene derivatives that affect transcriptional regulation by the estrogen receptors (ER $\alpha$  and ER $\beta$ ) but show different effects in different tissues. A third triphenylethylene derivative, GW-5638, displays tissue selectivity in rats identical to that of raloxifene, suggesting that GW-5638 and raloxifene share a mechanism of action that is different from that of tamoxifen.

**Results:** Both GW-5638 and its hydroxylated analog GW-7604 were tested for their ability to bind to ER $\alpha$  and ER $\beta$  and their ability to affect transcription of ER $\alpha$  and ER $\beta$  at a consensus estrogen response element and an ER/AP-1 response element. The drugs were found to have the same affinity for ER $\alpha$  and ER $\beta$ , although they were also found to activate transcription from an AP-1 promoter element more potently with ER $\beta$  than with ER $\alpha$ .

Derivatives of GW-5638 with alterations at the carboxylic acid still showed increased ER $\beta$  potency compared to ER $\alpha$ , but the magnitude of the activation with ER $\alpha$  was much higher than with ER $\beta$ .

**Conclusions:** Despite similar binding affinities to isolated ER $\alpha$  and ER $\beta$ , GW-5638 and GW-7604 show markedly lower EC<sub>50</sub> values with ER $\beta$  at an AP-1-driven promoter as compared to ER $\alpha$ . This suggests that the two compounds produce a more active ER/AP-1 conformation of the ER/AP-1 transcription factor complex when bound to ER $\beta$  than when bound to ER $\alpha$ . © 2001 Elsevier Science Ltd. All rights reserved.

**Keywords:** Estrogen receptor; GW-5638; Raloxifene; SERM; Tamoxifen; AP-1

## 1. Introduction

The estrogen receptors (ER $\alpha$  and ER $\beta$ ) are members of a large family of nuclear receptors that activate or repress transcription of genes in response to small molecule ligands [1]. Estrogen receptors play an important regulatory role in the reproductive, skeletal and cardiovascular systems and are validated therapeutic targets for diseases such as breast cancer and osteoporosis. A number of drugs have been developed that target the ER and many of these show different activities in different tissues [2]. For example, the breast cancer drug tamoxifen (**2**) functions as an antiestrogen in breast tissue, but mimics the activity of the physiological hormone, estradiol (**1**), in the uterus and bone. In contrast, the osteoporosis drug raloxifene (**3**) acts

as an antiestrogen in both breast and uterine tissue while being estrogenic in bone. (Structures of the compounds used in this study are shown in Fig. 1.)

One explanation for the different tissue effects of these drugs is that a ligand may elicit different responses when the receptor binds to different effector sites [3,4]. The estrogen receptor regulates transcription through binding to estrogen response elements (EREs) in the upstream promoter regions of target genes. At present, the most studied consensus ERE is the palindromic core sequence GGTCANNNTGACC which is recognized by homodimeric liganded ERs [5,6]. However, there is a growing body of evidence suggesting that there are important DNA effector sites for the estrogen receptors that differ significantly in sequence from the consensus ERE. These non-classical sites do not necessarily require protein–DNA interactions between the receptor and the promoter element, but instead can regulate transcription through protein–protein interactions between the receptor and other transcription factors, such as Sp-1 and AP-1 [7,8].

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Through receptor interactions with different response elements, the same ligand can cause activation or repression of different sets of genes. One of the most interesting features about these non-classical response elements is that antiestrogens can induce transcriptional activation through ERs at these sites.

A second variable to consider for tissue selectivity is that there are two different subtypes of the estrogen receptor, ER $\alpha$  and ER $\beta$ . The two subtypes share a high degree of sequence similarity in the regions of the receptor responsible for DNA binding and ligand binding, but differ substantially in the A/B and F regions of the receptor. It has been shown that the response to both estrogens and antiestrogens at an AP-1 site depends on the subtype of the receptor [9]; estradiol elicits transcriptional activation with ER $\alpha$ , but transcriptional repression with ER $\beta$ . The two ER subtypes also respond differently to raloxifene at an AP-1 site; ER $\beta$  shows much stronger activation than ER $\alpha$  in response to raloxifene. Subtype-selective activities have also been seen at other classical and non-classical estrogen response elements [7,10,11].

A triphenylethylene derivative, GW-5638 (**4**), has been reported to have tissue-selective effects similar to raloxifene, in that it is estrogenic in bone and antiestrogenic in breast and uterine tissue [12,13]. There are two interesting features about GW-5638: (1) it shows a tissue profile similar to raloxifene despite having the ethyltriphenylethylene scaffold of tamoxifen, and (2) GW-5638 contains a carboxylic acid on the side chain extending from the central ethyltriphenylethylene core which differs from the amine-containing side chains of both tamoxifen and raloxifene.

To date, there have been no reports of induction of transcription by GW-5638; only antiestrogenic *in vitro* activities have been reported with this compound. Here, we show that GW-5638 can induce ER-dependent transcriptional activation at an AP-1 site and that it does so in an ER $\beta$ -selective way. We also show that the negative charge of the carboxylic acid side chain is an essential feature of this ER $\beta$ -selective response.

## 2. Results

### 2.1. Synthesis of GW-5638 and derivatives

Both GW-5638 and its more potent, hydroxylated analog, GW-7604, were synthesized following a published synthetic route for GW-5638 [12]. In the case of GW-7604, which contains a hydroxyl group on one of the phenyl rings, the synthesis was modified to carry through the hydroxyl group protected as a *t*-butyl ether (Scheme 1). While the synthesis of GW-5638 produces only the desired *Z* double bond geometry at the triphenylethylene core, the synthesis of GW-7604 produces a mixture of *Z* and *E* isomers. The olefin isomerization occurs in the bromination step that provides bromide **11**. We investigated a variety of alternative conditions to prevent this isomerization but all attempts were unsuccessful. However, we were able to find conditions to precipitate the undesired *Z* isomer of GW-7604 such that preparations of pure *Z* and *E/Z* mixtures could be obtained. A Horner–Emmons reaction was used on an intermediate in the synthesis of

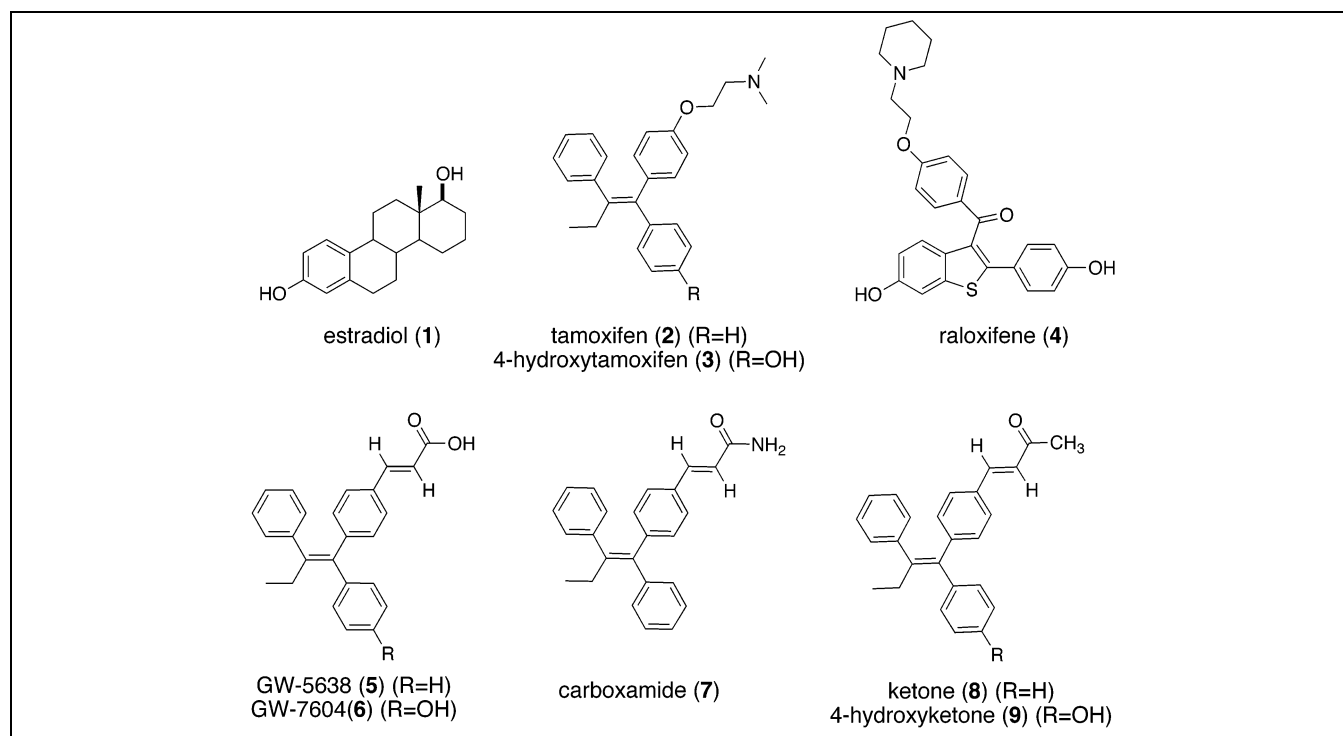
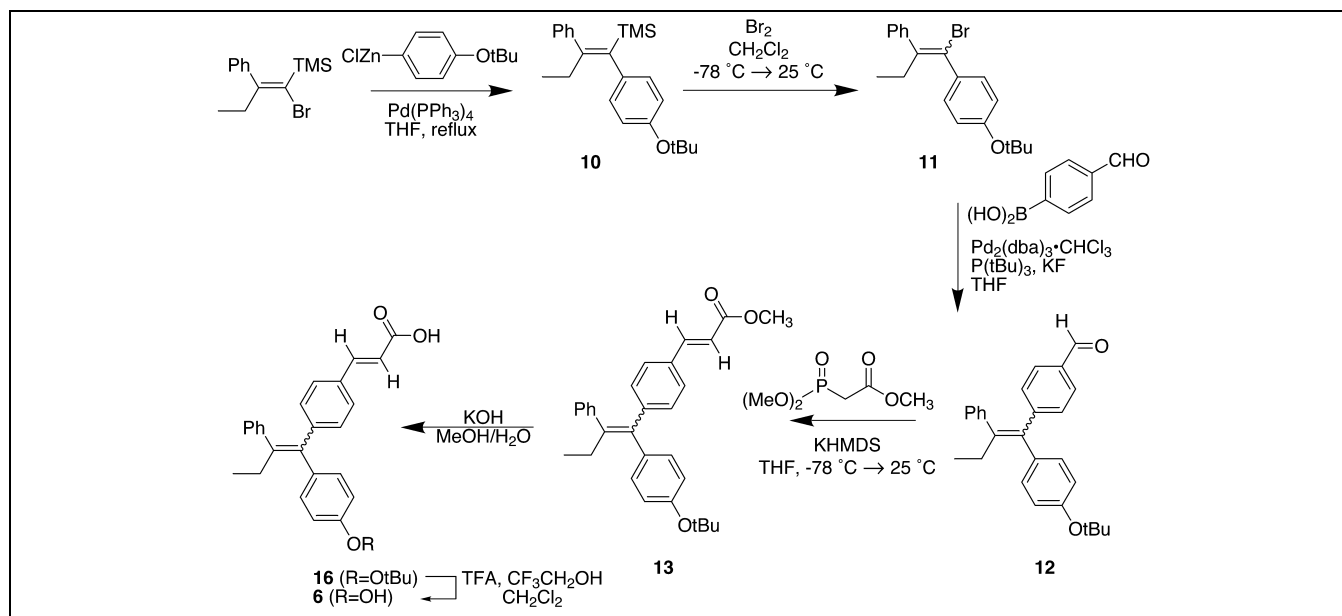


Fig. 1. Compounds used in this study.



Scheme 1.

GW-5638 and GW-7604 (Scheme 2) to make analogs where the carboxylic acid was replaced with a methyl ketone (**8** and **9**). The synthesis of the methyl ketone derivative of GW-7604 produced a mixture of *Z* and *E* isomers that were inseparable.

## 2.2. Ligand binding to ER $\alpha$ and ER $\beta$

All compounds were tested for binding affinity to ER $\alpha$  and ER $\beta$  in a competition assay with radiolabeled estradiol (Table 1). GW-5638 and GW-7604 did not show binding selectivity between ER $\alpha$  and ER $\beta$ ; GW-5638 bound to both ERs with 5–8% of the affinity of estradiol, whereas GW-7604 bound to both ERs with 15% of the affinity of estradiol. The methyl ketone derivatives **8** and **9** had roughly 5–10-fold lower binding affinity for ER $\alpha$  and ER $\beta$  compared to their carboxyl analogs suggesting that the acid moiety is involved in an important binding contact. One of the well-documented difficulties of working

with the 4-hydroxylated tamoxifen derivatives is the facile *E/Z* isomerization, particularly in the cell-based assays used to measure activity [14,15]. Compared to the *E/Z* mixture, the pure *Z* isomer of GW-7604 shows 8-fold lower affinity for ER $\alpha$  and 15-fold lower affinity for ER $\beta$ . A similar difference was seen between the two isomers of hydroxytamoxifen in binding to ER $\alpha$  [15]. In that case, isolation of hydroxytamoxifen from estrogen receptors in cell-based assays resulted in recovery of only the higher affinity form [15]. With a similar difference in binding affinity for the two isomers of GW-7604, any activity seen in cell-based assays with the *E/Z* mixture can be attributed to the *E* isomer since it will be the predominant isomer that is bound to the receptor.

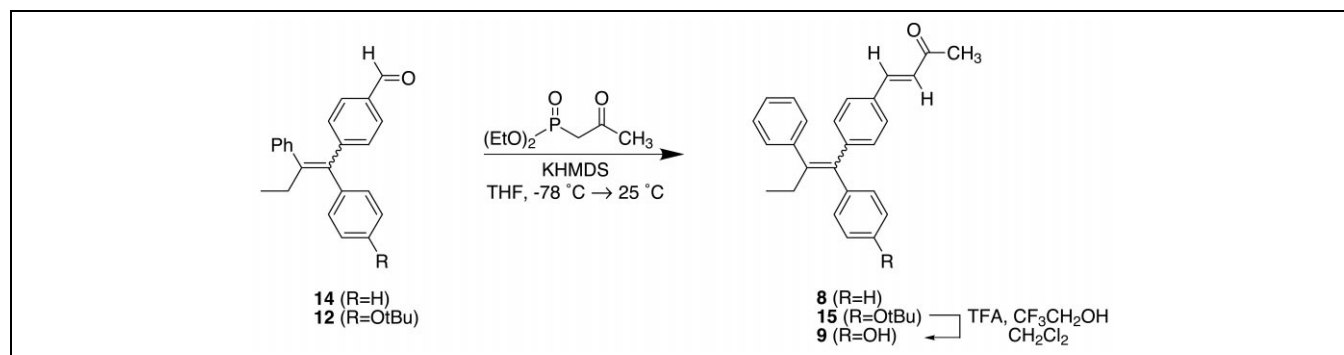
## 2.3. Ligand activation at ERE and AP-1 sites

GW-5638 and GW-7604 were then tested for their effects on ER-mediated transcription in HeLa cells using

Table 1  
Relative binding affinities (RBA) of ligands to full length ER $\alpha$  or ER $\beta$  calculated as described in Section 5

Ligand	RBA	
	ER $\alpha$	ER $\beta$
Estradiol ( <b>1</b> )	100	100
4-Hydroxytamoxifen ( <b>3</b> )	36	43
Raloxifene ( <b>4</b> )	34	76
GW-5638 ( <b>5</b> )	5	8
GW-7604 ( <b>6</b> ) ( <i>Z</i> and <i>E</i> isomers)	15	15
GW-7604 ( <i>Z</i> isomer)	2	1
Ketone ( <b>8</b> )	1.3	0.2
4-Hydroxyketone ( <b>9</b> ) ( <i>Z</i> and <i>E</i> isomers)	2.4	1

Relative binding affinities are expressed as a percentage of the potency of estradiol. Under the experimental conditions described in Section 5, estradiol was found to have an  $\text{IC}_{50}$  of 5 nM for ER $\alpha$  and 3 nM for ER $\beta$ .



Scheme 2.

reporter gene assays. The two reporter gene constructs were regulated either by a consensus estrogen response element (ERE) mentioned above, or by an AP-1 response element from the upstream region of the collagenase gene. In our studies, a G400V point mutant of ER $\alpha$  was used. This mutant, called HE0, shows lower ligand-independent activity and has been described previously with the AP-1 response element [9]. In our present studies, as before, the ligand-dependent activity of HE0 was no different from wild-type ER $\alpha$  for any of the compounds tested. With an ERE-driven luciferase reporter, GW-5638 and GW-7604 antagonized an estradiol response with ER $\alpha$  and ER $\beta$  (Fig. 2), in agreement with previous reports [13]. With an AP-1-driven luciferase reporter, differences in ligand activation between ER $\alpha$  and ER $\beta$  were seen with GW-5638 and GW-7604. Dose–response curves for GW-5638 and GW-7604 show that the two compounds activate transcription with ER $\beta$  more potently at the AP-1 site than with ER $\alpha$ ; the EC<sub>50</sub> value of induction with ER $\beta$  is at least 15 times more potent than with ER $\alpha$  for GW-5638 and approximately 50 times more potent for GW-7604 (Fig. 3). In contrast, 4-hydroxytamoxifen and ralox-

ifene induce responses with ER $\alpha$  and ER $\beta$  that differ only in the magnitude of the transactivation. Tamoxifen induces a stronger response with ER $\alpha$  than with ER $\beta$  and raloxifene induces a stronger response with ER $\beta$  than with ER $\alpha$ , but both compounds have similar EC<sub>50</sub> values for reporter induction. To our knowledge, this is the first report of ER-mediated induction of transcriptional activation by GW-5638 or GW-7604.

When the carboxamide (7) and the ketone analogs (8 and 9) were tested in transactivation assays at an ERE site, all three compounds were found to be antagonists of estradiol activation with both ER $\alpha$  and ER $\beta$ . However at the AP-1 site, agonism was observed and the carboxamide (7) and 4-hydroxyketone (9) showed much stronger activation with ER $\alpha$  than with ER $\beta$  (Fig. 4). In a dose–response experiment, the hydroxyketone (9) was found to activate ER $\beta$  more potently than ER $\alpha$ , yet showed a much higher magnitude of activation with ER $\alpha$  than with ER $\beta$ . The higher level of activation with ER $\alpha$  compared to ER $\beta$  resembles the profile of 4-hydroxytamoxifen rather than that of GW-5638 and GW-7604 (Fig. 3).

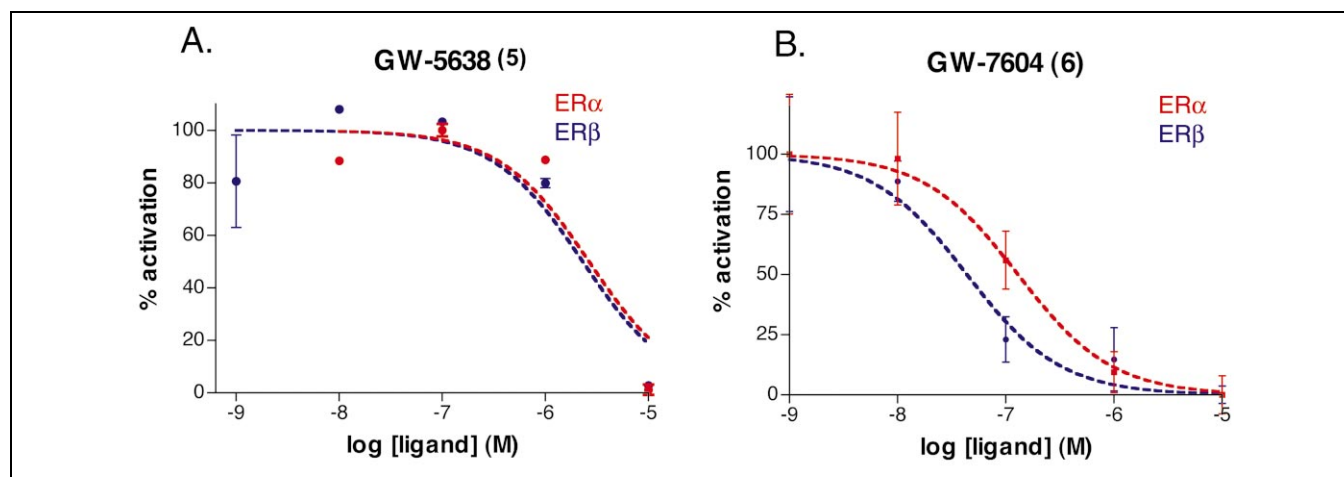


Fig. 2. Competition of (A) GW-5638 (5) and (B) GW-7604 (6) versus 0.1 nM estradiol in transient transfection assay of HeLa cells with ER $\alpha$  (orange) and ER $\beta$  (purple) and the vitellogenin A2 ERE-tk-driven luciferase reporter gene. Curve represents the best fit to a single-site competition binding model. 100% activation represents the activation with 0.1 nM estradiol alone.

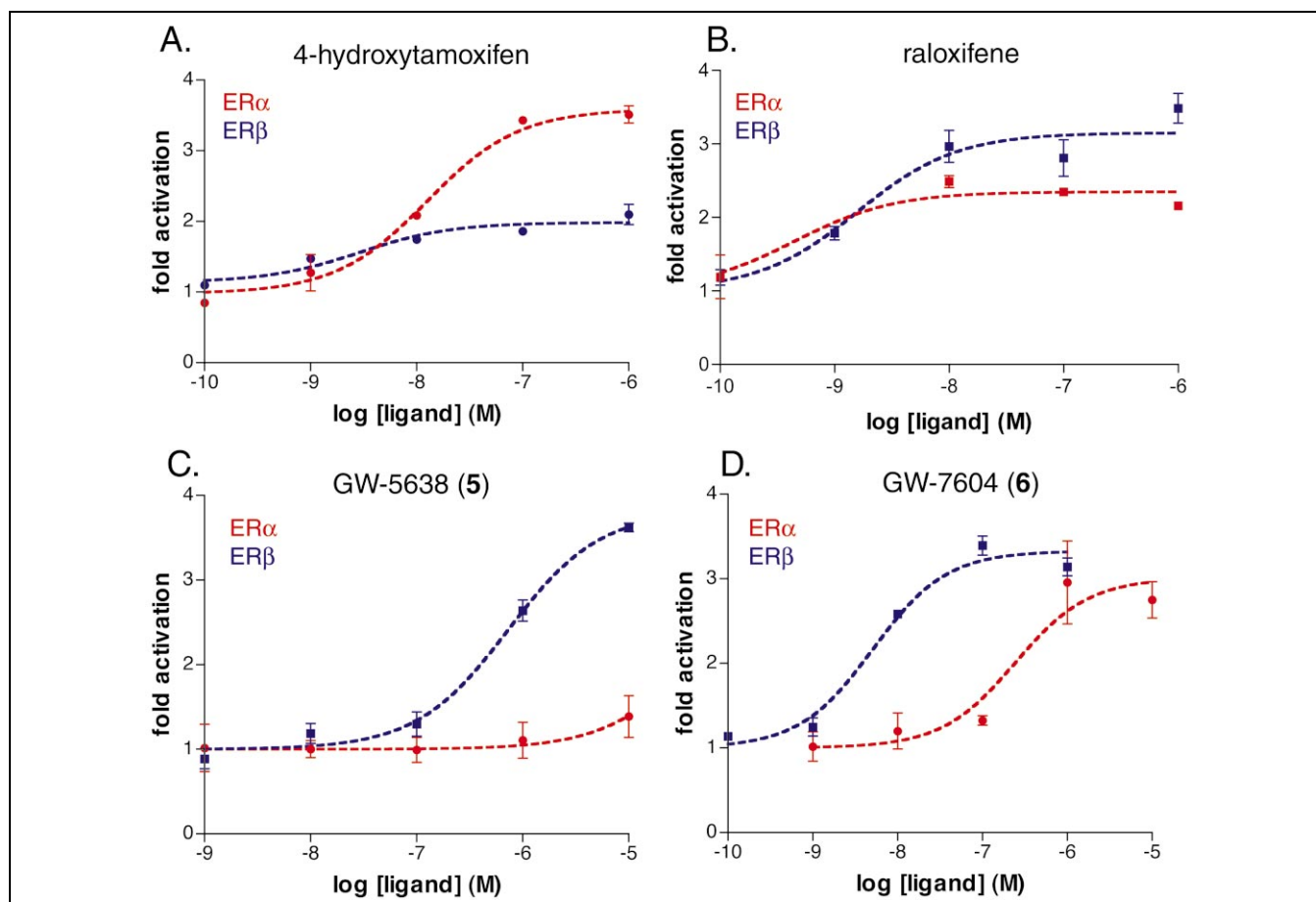


Fig. 3. Dose-response curves for transient transfections with the AP-1-driven luciferase reporter gene using (A) 4-hydroxytamoxifen, (B) raloxifene, (C) GW-5638 (5), and (D) GW-7604 (6). Data for transfections with ER $\alpha$  shown in orange and data for ER $\beta$  shown in purple. Fold activation determined relative to the response with no hormone present. Curve represents best fit of data using a single-site sigmoidal dose-response model.

### 3. Discussion

#### 3.1. Mechanism of ER $\beta$ -selective signaling at the AP-1 site

Ligand binding experiments show that there is no ER $\alpha$ /ER $\beta$  binding selectivity for GW-5638, GW7604 or ketone derivatives **8** and **9** (Table 1). These compounds have a weaker binding affinity than 4-hydroxytamoxifen and raloxifene, but like 4-hydroxytamoxifen and raloxifene, the affinity is approximately the same with ER $\alpha$  and ER $\beta$ . Furthermore, competition experiments at the ERE site

show that these compounds have similar IC<sub>50</sub> values with ER $\alpha$  and ER $\beta$  for antagonism of an estradiol response, a property that is again similar to 4-hydroxytamoxifen and raloxifene (Table 2). However, the properties of the compounds at the AP-1 site differ from those of 4-hydroxytamoxifen and raloxifene. All of the compounds are agonists of transactivation at AP-1 and show greater potency with ER $\beta$  than with ER $\alpha$  (Table 2). GW-7604 is approximately 50 times more potent at inducing activation with ER $\beta$  than with ER $\alpha$ . This differs from 4-hydroxytamoxifen and raloxifene, which show similar potencies with

Table 2

Comparison of ligand activities in competition experiments at an ERE-driven reporter gene and in activation experiments at an AP-1-driven reporter gene

	IC <sub>50</sub> at ERE		EC <sub>50</sub> at AP-1 site	
	ER $\alpha$	ER $\beta$	ER $\alpha$	ER $\beta$
GW-5638	2.6 $\mu$ M	3.8 $\mu$ M	> 10 $\mu$ M	730 nM
GW-7604	130 nM	43 nM	240 nM	5.1 nM
Ketone ( <b>8</b> )	5.6 $\mu$ M	1.2 $\mu$ M	> 10 $\mu$ M	> 10 $\mu$ M
4-Hydroxyketone ( <b>9</b> )	50 nM	15 nM	2.2 $\mu$ M	110 nM

Competition experiments performed versus 0.1 nM estradiol. IC<sub>50</sub> values determined using a single binding site competition model. EC<sub>50</sub> values determined using a single binding site sigmoidal dose-response model.

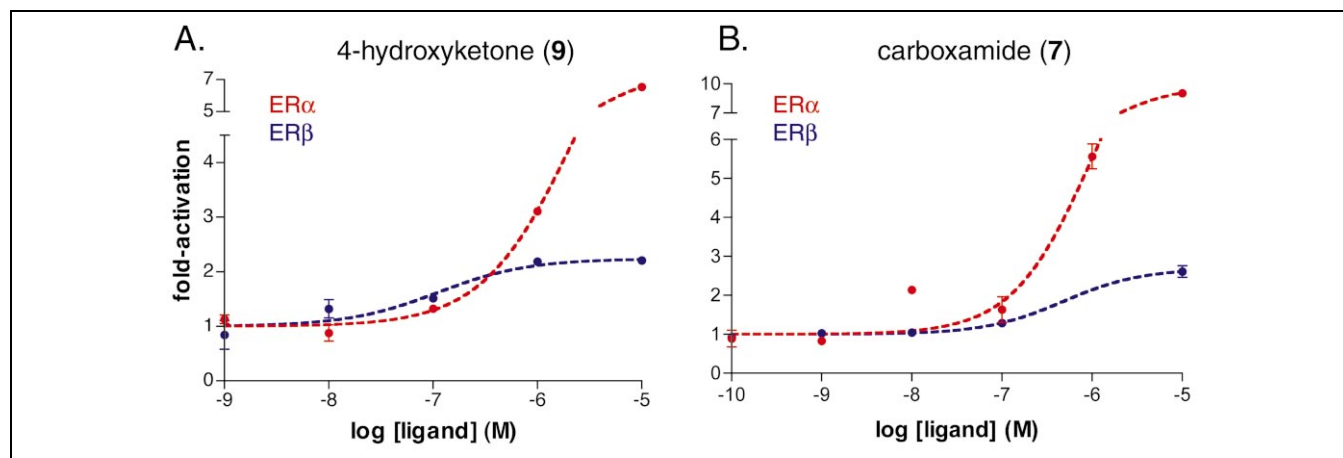


Fig. 4. Dose-response curves for transient transfections with the AP-1-driven luciferase reporter gene using 4-hydroxyketone (9) and carboxamide (7). Data for transfections with ERα shown in orange and data for ERβ shown in purple. Fold activation determined relative to the response with no hormone present. Curve represents best fit of data using a single-site sigmoidal dose-response model.

ERα and ERβ but different levels of maximum induction (Fig. 3). The 4-hydroxyketone derivative (9) shows differences between ERα and ERβ in both the potency and the level of maximum induction. Taken together, these results indicate an unusual situation in which ligand binding affinity for the two estrogen receptors of GW-5638 and GW-7604 is not directly connected to potency of ligand induction at an AP-1 site.

This suggests that there may be different mechanisms of ligand induction for ERα and ERβ at AP-1 sites. Ligand binding by the estrogen receptor is merely the first step in a pathway that leads to transcriptional activation. Other accessory proteins are known to interact with the receptor after ligand is bound to form a transcriptionally active complex [16]. The fact that GW-5638 and GW-7604 as well as their ketone derivatives (8 and 9) are able to activate transcription at the AP-1 site more potently with ERβ compared to ERα suggests that the accessory proteins necessary for activation are recruited more efficiently by liganded ERβ than by liganded ERα. GW-5638 and GW-7604 bind to ERβ to produce a strongly active ER/AP-1 whereas the same two drugs bind to ERα to produce a weakly active ER/AP-1 conformation. It is important to note that in this situation, the affinity of the ligand for each receptor subtype is less important in determining the overall activity of the compound than the ability of the ligand–receptor complex to transduce signals to the basal transcription machinery.

The work reported here demonstrates that compounds without binding selectivity for ERα and ERβ can still show significant ERα/ERβ selectivity in ligand induction. Mutational analysis suggests that domains in addition to the ligand binding domain are important in binding putative accessory proteins involved in the AP-1 response [17]. This means that ligand binding can cause different inter-domain interactions in ERα compared to ERβ and that this may be the underlying explanation for the differences

in the AP-1 activity of the two subtypes in response to different ligands. In support of this model, interchanging the highly different N-terminal domains of ERα and ERβ has a dramatic effect on the response of the two subtypes to tamoxifen and raloxifene at the AP-1 site [18].

### 3.2. Correlating SERM activity with the AP-1 response

A signature feature of compounds that target the estrogen receptor is that they often show different effects in different tissues. For instance tamoxifen has antiestrogenic properties in breast tissue but estrogenic properties in uterine and bone tissue. In contrast, raloxifene is antiestrogenic in uterine tissue while still maintaining estrogenic activity in bone. At the AP-1 site, raloxifene shows greater activation with ERβ compared to ERα whereas tamoxifen shows greater activation with ERα. The possibility that the activation of transcription at ERα via the AP-1 site might be correlated with the estrogenic properties in uterine tissue is further strengthened by this work. GW-5638 shows greater ERβ activation at the AP-1 site while the carboxamide analog (7) shows greater activation with ERα. This can be correlated to the original report on the compounds; GW-5638 lacked uterotrophic activity in rats whereas estrogenic activity in the uterus was seen with the carboxamide derivative [12].

## 4. Significance

This work further demonstrates that the estrogen receptor (ER) subtype selectivity of a given ligand at an AP-1 site cannot be determined solely by binding affinity to ERα and ERβ. In addition, a correlation is established between the tissue estrogenic profile of a panel of selective estrogen receptor modulators (SERMs) and their activation profile in an ER-mediated transcriptional assay.

## 5. Materials and methods

### 5.1. General procedures

The construction of the expression vectors for both hER $\alpha$  (HE0) and hER $\beta$  as well as the AP-1 regulated luciferase construct ( $\Delta$ Coll73-luc) have been described [8,9]. The ERE-driven luciferase reporter gene consists of two repeats of the upstream region of the vitellogenin ERE promoter from -331 to -289, followed by region -109 to +45 of the thymidilate kinase upstream region and the luciferase gene. Whereas a point mutant of ER $\alpha$  (HE0) that has a lower hormone-independent response was used in these studies, previous experiments have shown that there are no differences in the responses of the HE0 mutant and the wild-type ER $\alpha$  at the AP-1 site [9]. The longer form of ER $\beta$  with 149 residues in the N-terminal domain was used in transfection experiments. No significant differences in the activation at either the ERE or AP-1 site were observed with the shorter version of ER $\beta$  with 96 residues (data not shown). Proton and carbon-13 nuclear magnetic resonance spectra ( $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR) were obtained on a Varian INOVA-400 (400 MHz) instrument;  $^1\text{H}$  NMR chemical shifts are reported as  $\delta$  values in parts per million (ppm) downfield from internal tetramethylsilane, or downfield from the residual  $\text{H}_2\text{O}$  peak in  $\text{CD}_3\text{OD}$ ;  $^{13}\text{C}$  NMR chemical shifts are reported as  $\delta$  values with reference to the solvent peak. High-resolution mass spectrometry (HRMS) was performed by the National Bioorganic and Biomedical Mass Spectrometry Resource at UCSF.

### 5.2. Tissue culture, transfection and luciferase assays

HeLa cells were grown in 0.1  $\mu\text{m}$  filtered DME supplemented with 4.5 g/l glucose, 0.876 g/l glutamine, 100 mg/l streptomycin sulfate, 100 units/ml of penicillin G and 10% newborn calf serum. Cells were grown to a density of not more than  $5 \times 10^4$  cells per  $\text{cm}^2$ . For transient transfection assays, cells were suspended in 0.5 ml electroporation buffer in 0.4 cm gap electroporation cuvettes at approximately  $1.5 \times 10^6$  cells per cuvette with 5  $\mu\text{g}$  of the reporter plasmid and the optimal amount of the receptor expression vector for ligand activation, determined previously to be 5  $\mu\text{g}$  plasmid per transfection for the AP-1 response or 1  $\mu\text{g}$  expression plasmid per transfection for the ERE response. The electroporation buffer consisted of 0.2  $\mu\text{m}$  filtered PBS and 0.1% glucose. Cells were transfected by electroporation at a potential of 0.25 kV and a capacitance of 960 mF. Transfected cells were pooled and immediately resuspended in growth media supplemented as described above with the exception that the newborn calf serum had been treated with charcoal as described previously [9]. Cells were plated into 6- or 12-well dishes at 2 ml per well at a density of approximately  $1 \times 10^5$  cells per well. After 2 h of incubation at 37°C, hormones were added in 2  $\mu\text{l}$  of ethanol.

After 24 h of incubation at 37°C, the cells were lysed by first removing the media from the wells, washing with PBS and then adding 0.2 ml of lysis buffer consisting of 100 mM potassium phosphate (pH 7.5), 0.2% Triton X-100 and 1 mM DTT. After 10 min at room temperature, 0.1 ml of the lysate was combined

with 0.3 ml of the luciferase assay buffer consisting of 25 mM glycylglycine, 15 mM  $\text{MgSO}_4$ , 4 mM EGTA, 15 mM potassium phosphate (pH 7.8) with the addition to a final concentration of 1 mM DTT, 2 mM ATP and 0.2 mM luciferin. Luminescence was measured for 10 s with a Monolight 3010 luminometer (Analytical Luminescence Laboratory, San Diego, CA, USA). Each hormone dose was performed in triplicate and the relative error was determined by calculating the standard error of the three values from the mean. Experiments were conducted multiple times to ensure reproducibility of the results.

### 5.3. ER binding assays

The relative binding affinity of compounds for ER $\alpha$  and ER $\beta$  was determined using a spin column assay with commercially available full length forms of both ER $\alpha$  and ER $\beta$  (PanVera Corp, Madison, WI, USA). Receptor was added to a final concentration of 15 nM to a solution containing 10 mM Tris, pH 7.5, 10% glycerol, 2 mM DTT and 1 mg/ml BSA and 3 nM [2,4,6,7,16,17- $^3\text{H}$ ]estradiol at 4°C. 100  $\mu\text{l}$  of the solution was added to 1  $\mu\text{l}$  of the ligand in ethanol, mixed gently by pipetting and incubated at 4°C overnight. The mixture was then applied to a micro spin column containing G-25 Sephadex (Harvard Apparatus Inc.) equilibrated in binding buffer (minus tritiated estradiol) according to the manufacturer's instructions. Bound estradiol was separated from free ligand by spinning at  $2000 \times g$  for 4 min at room temperature. The filtrate was then added to 2.5 ml of scintillant and counted in a liquid scintillation counter. Each point in the binding curve represents the average of two separate experiments and the curve was fit using a single binding site competition model with the Prism statistical analysis software package. The standard deviation was determined to be less than 0.2 log units from the  $\text{EC}_{50}$  value. Percent relative binding affinity was then determined by dividing the  $\text{IC}_{50}$  determined for unlabeled estradiol by the ligand  $\text{IC}_{50}$  and multiplying that by 100.

### 5.4. Synthesis of compounds

#### 5.4.1. (3E)-3-[4-[(1Z)-1,2-Diphenyl-1-butenyl]phenyl]-3-buten-2-one (**8**)

A solution of potassium bis(trimethylsilyl)amide (0.5 M in toluene) (253  $\mu\text{l}$ , 0.13 mmol) was added to a stirring 0°C solution of diethyl (2-oxopropyl)phosphonate (24  $\mu\text{l}$ , 0.13 mmol) in tetrahydrofuran (0.8 ml). After stirring for 15 min at 0°C, the solution was cooled to -78°C, and a solution of 1,2-diphenyl-1-(4-formylphenyl)-but-1-ene (33 mg, 0.11 mmol) (**14**) [12] in tetrahydrofuran (0.4 ml) was added dropwise. The reaction was stirred at -78°C for 5 min, then allowed to warm to room temperature and stirred 20 h overnight. The solution was poured into saturated sodium chloride, extracted with ethyl acetate, dried over anhydrous magnesium sulfate, and solvent removed under reduced pressure. The crude oil was purified by flash column chromatography (0–5% ethyl acetate–hexanes) to give **8** as a yellow oil (13.3 mg, 0.038 mmol) in 36% yield:  $R_f$  0.26 (10% ethyl acetate–hexanes);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.94 (t,  $J$  = 7.6 Hz, 3 H), 2.31 (s, 3 H), 2.48 (q,  $J$  = 7.6 Hz, 2 H), 6.56 (d,  $J$  = 16 Hz, 1 H), 6.90



(d,  $J=8.0$  Hz, 2 H), 7.11–7.38 (m, 13 H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ) 13.47, 27.50, 29.00, 127.43, 127.47, 127.84, 127.99, 128.16, 128.28, 128.57, 129.49, 129.58, 130.16, 130.76, 131.34, 138.05, 141.78, 143.21, 143.36, 198.41 ppm; HRMS (EI) exact mass calculated for  $\text{C}_{26}\text{H}_{24}\text{O}$ : 352.1827, found: 352.1826.

#### 5.4.2. *Z*-2-Phenyl-1-(4-*tert*-butoxyphenyl)-1-trimethylsilyl-but-1-ene (**10**)

A solution of lithium 4-*tert*-butoxybenzene was prepared by adding *n*-butyl lithium (2.5 M in hexanes) (3.17 ml, 7.94 mmol) to a  $-78^\circ\text{C}$  solution of 4-*tert*-butoxybromobenzene (1.81 g, 7.94 mmol) in tetrahydrofuran (20 ml). The reaction was stirred at  $-78^\circ\text{C}$  for 30 min. In a separate flask, anhydrous zinc chloride (1.04 g, 7.94 mmol) was dissolved in tetrahydrofuran (23 ml) and a solution of 4-*tert*-butoxyphenylzinc chloride was prepared by adding the lithium 4-*tert*-butoxybenzene solution at such a rate that a slow reflux was maintained. The solution was refluxed an additional 30 min, then cooled to room temperature. Tetrakis-(triphenylphosphine)palladium(0) (0.13 mmol) was added to a solution of (*E*)-1-bromo-2-phenyl-1-trimethylsilyl-1-butene (1.5 g, 5.29 mmol) in tetrahydrofuran (6 ml) and stirred at  $25^\circ\text{C}$  for 5 min. The 4-*tert*-butoxyphenyl zinc chloride solution was then added and the reaction was refluxed 17 h overnight. The solution was cooled to room temperature, quenched with 3 N hydrochloric acid, extracted with hexanes, washed with saturated sodium bicarbonate and dried over anhydrous magnesium sulfate. Solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (silica, 0–4% ether–hexanes) to afford **10** as a white crystalline solid (1.61 g, 4.56 mmol) in 86% yield:  $R_f$  0.58 (10% ethyl acetate–hexanes);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.36 (s, 9 H), 0.72 (t,  $J=7.6$  Hz, 3 H), 1.36 (s, 9 H), 2.15 (q,  $J=7.6$  Hz, 2 H), 6.89 (d,  $J=8.4$  Hz, 2 H), 6.95 (d,  $J=8.4$  Hz, 2 H), 7.21 (d,  $J=6.4$  Hz, 2 H), 7.27–7.34 (m, 3 H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ) 0.10, 12.80, 28.89, 30.05, 78.03, 123.80, 126.68, 127.66, 128.37, 128.98, 139.64, 140.50, 144.00, 152.67, 155.52 ppm; HRMS (EI) exact mass calculated for  $\text{C}_{23}\text{H}_{32}\text{OSi}$ : 352.2222, found: 352.2240.

#### 5.4.3. *Z*-1-Bromo-2-phenyl-1-(4-*tert*-butoxyphenyl)-but-1-ene (**11**)

To a solution of *Z*-2-phenyl-1-(4-*tert*-butoxyphenyl)-1-trimethylsilyl-but-1-ene (**10**) [19,20] (76.5 mg, 0.22 mmol) in dichloromethane (1 ml) at  $-78^\circ\text{C}$ , was added dropwise a 1 M solution of bromine in dichloromethane, until the reaction was complete as monitored by thin layer chromatography. The yellow solution was poured into 10% sodium sulfite, extracted with dichloromethane, washed with saturated sodium chloride, dried over anhydrous magnesium sulfate and solvent removed under reduced pressure to give an orange oil. The crude product was purified by flash column chromatography (0–5% ethyl acetate–hexanes) to give **11** as a yellow oil (53.9 mg, 0.15 mmol) in 69% yield, determined by  $^1\text{H}$  NMR to be a 3:2 mixture of *Z*:*E* isomers:  $R_f$  0.61 (20% ethyl acetate–hexanes);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.79 (t,  $J=7.6$  Hz, 3 H, *Z* isomer), 0.96 (t,  $J=7.6$  Hz, 2 H, *E* isomer), 1.19 (s, 6 H, *E* isomer), 1.31 (s, 9 H, *Z* isomer), 2.27 (q,  $J=7.6$  Hz, 2 H, *Z* isomer), 2.72 (q,  $J=7.6$  Hz, 1.3 H *E* isomer), 6.61 (d,  $J=8.8$

Hz, 1 H), 6.90–7.00 (m, 6 H), 7.17–7.26 (m, 6 H), 7.30–7.32 (m, 2 H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ) 11.68, 13.06, 28.77, 28.90, 29.50, 33.03, 78.63, 78.81, 118.74, 120.50, 122.99, 123.42, 126.57, 127.10, 127.83, 128.13, 128.30, 129.11, 129.61, 130.80, 135.48, 135.95, 140.54, 142.57, 144.03, 144.54, 154.52, 155.39 ppm; HRMS (EI) exact mass calculated for  $\text{C}_{20}\text{H}_{23}\text{OBr}$ : 360.0912, found: 360.0977.

#### 5.4.4. *E*-2-Phenyl-1-(4-*tert*-butoxyphenyl)-1-(4-formylphenyl)-but-1-ene (**12**)

To a flask containing a 1-bromo-2-phenyl-1-(4-*tert*-butoxyphenyl)-but-1-ene (**11**) isomer mixture (91.2 mg, 0.25 mmol) was added 4-formylphenylboronic acid (41.9 mg, 0.28 mmol), potassium fluoride (48.7 mg, 0.84 mmol) and tris(dibenzylideneacetone)dipalladium(0)–chloroform adduct (1.31 mg, 0.0013 mmol). After dissolving in tetrahydrofuran (1 ml), a 0.12 M solution of tri-*tert*-butylphosphine (0.62 mg, 0.003 mmol) in tetrahydrofuran was added [21]. The reaction turned from red to brown in 15 min, and was then stirred at  $25^\circ\text{C}$  for 16 h overnight, by which time it had turned a clear yellow with a black residue. The solution was poured into saturated sodium bicarbonate, extracted with ethyl acetate, dried over anhydrous magnesium sulfate, and solvent removed under reduced pressure. The crude oil was purified by flash column chromatography (0–6% ether–hexanes) to give **12** as a yellow oil (43.8 mg, 0.11 mmol) in 46% yield, determined by  $^1\text{H}$  NMR to be a 2:1 mixture of *E*:*Z* isomers:  $R_f$  0.52 (20% ethyl acetate–hexanes);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.95 (t,  $J=7.6$  Hz, 4.5 H), 1.24 (s, 4.5 H, *Z* isomer), 1.38 (s, 9 H, *E* isomer), 2.46 (q,  $J=7.6$  Hz, 1 H, *Z* isomer), 2.52 (q,  $J=7.6$  Hz, 2 H, *E* isomer), 6.64 (d,  $J=8.4$  Hz, 1 H), 6.72 (d,  $J=8.4$  Hz, 1 H), 6.98 (d,  $J=8.4$  Hz, 2 H), 7.03–7.16 (m, 11.5 H), 7.44 (d,  $J=8.0$  Hz, 1 H), 7.52 (d,  $J=8.0$  Hz, 2 H), 7.88 (d,  $J=8.0$  Hz, 1 H), 9.83 (s, 1 H, *E* isomer), 10.03 (s, 1 H, *Z* isomer);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ) 13.44, 13.50, 28.75, 28.81, 28.90, 29.14, 78.31, 78.52, 123.16, 123.69, 126.38, 126.61, 127.81, 128.02, 128.87, 129.53, 129.55, 129.68, 130.02, 130.19, 131.18, 131.39, 133.71, 134.76, 137.12, 137.27, 137.46, 137.56, 141.58, 141.62, 143.21, 144.38, 150.04, 150.18, 153.60, 154.50, 191.91, 191.94 ppm; HRMS (EI) exact mass calculated for  $\text{C}_{27}\text{H}_{28}\text{O}_2$ : 384.2089, found: 384.2090.

#### 5.4.5. (3*E*)-3-[4-[(1*E*)-2-Phenyl-1-(4-*tert*-butoxyphenyl)-1-butenyl]phenyl]-3-buten-2-one (**15**)

A solution of potassium bis(trimethylsilyl)amide (0.5 M in toluene) (247  $\mu\text{l}$ , 0.12 mmol) was added to a stirring  $0^\circ\text{C}$  solution of diethyl (2-oxopropyl)phosphonate (47.5  $\mu\text{l}$ , 0.12 mmol) in tetrahydrofuran (0.8 ml). After stirring for 15 min at  $0^\circ\text{C}$ , the solution was cooled to  $-78^\circ\text{C}$ , and a solution of 2-phenyl-1-(4-*tert*-butoxyphenyl)-1-(4-formylphenyl)-but-1-ene (**12**) isomer mixture (39.6 mg, 0.10 mmol) in tetrahydrofuran (0.4 ml) was added dropwise. The reaction was stirred at  $-78^\circ\text{C}$  for 5 min, then allowed to warm to room temperature and stirred 20 h overnight. The solution was poured into saturated sodium chloride, extracted with ethyl acetate, dried over anhydrous magnesium sulfate, and solvent removed under reduced pressure. The crude oil was purified by flash column chromatography (0–10% ethyl acetate–hexanes) to give **15** as a yellow oil (40.3 mg, 0.095 mmol) in 92% yield, determined by  $^1\text{H}$  NMR to be a 3:2 mixture of *E*:*Z* isomers (at



the triphenylethylene center):  $R_f$  0.46 (30% ethyl acetate–hexanes);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.94 (t,  $J$  = 7.6 Hz, 3 H, *E* isomer), 0.95 (t,  $J$  = 7.6 Hz, 2 H, *Z* isomer), 1.24 (s, 6 H, *Z* isomer), 1.38 (s, 9 H, *E* isomer), 2.31 (s, 3 H, *E* isomer), 2.39 (s, 2 H, *Z* isomer), 2.49 (q,  $J$  = 7.6 Hz, 1.7 H, *Z* isomer), 2.50 (q,  $J$  = 7.2 Hz, 2 H, *E* isomer), 6.56 (d,  $J$  = 16.4 Hz, 1 H, *E* isomer), 6.62–7.31 (m, 21.7 H), 7.35 (d,  $J$  = 16.4 Hz, 1 H, *E* isomer), 7.51–7.55 (m, 1.3 H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ) 13.47, 13.60, 27.35, 27.51, 28.75, 28.80, 28.90, 29.13, 78.24, 78.44, 123.10, 123.63, 126.20, 126.38, 126.75, 127.39, 127.75, 127.95, 128.14, 129.59, 130.01, 130.16, 131.20, 131.40, 131.62, 132.68, 137.55, 137.73, 137.71, 141.88, 141.98, 142.67, 143.23, 143.38, 145.96, 146.16, 153.44, 154.34, 198.35, 198.38 ppm; HRMS (EI) exact mass calculated for  $\text{C}_{30}\text{H}_{32}\text{O}_2$ : 424.2402, found: 424.2406.

#### 5.4.6. (3*E*)-3-[4-[(1*E*)-2-Phenyl-1-(4-hydroxyphenyl)-1-butenyl]phenyl]-3-buten-2-one (**9**)

To a solution of a (3*E*)-3-[4-[2-phenyl-1-(4-*tert*-butoxyphenyl)-1-butenyl]phenyl]-3-buten-2-one (**15**) isomer mixture (39.0 mg, 0.92 mmol) in dichloromethane (2 ml) was added trifluoroacetic acid (100  $\mu\text{l}$ ) and 2,2,2-trifluoroethanol (0.5 ml). The reaction was stirred 12 h overnight at 25°C, after which the solution was poured into water, extracted with diethyl ether, washed with saturated sodium bicarbonate, and the solvent removed under reduced pressure. The crude yellow solid was purified by preparative thin layer chromatography (30% ethyl acetate–hexanes, eluted off silica with ethyl acetate), to afford **9** as a yellow solid (25.7 mg, 0.070 mmol) in 76% yield, determined by  $^1\text{H}$  NMR to be a 3:2 mixture of *E*:*Z* isomers (at the triphenylethylene center):  $R_f$  0.40 (30% ethyl acetate–hexanes);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.87 (t,  $J$  = 7.6 Hz, 5 H), 2.25 (s, 3 H, *E* isomer), 2.32 (s, 2 H, *Z* isomer), 2.42 (q,  $J$  = 7.6 Hz, 1.7 H, *Z* isomer), 2.44 (q,  $J$  = 7.6 Hz, 2 H, *E* isomer), 6.42 (d,  $J$  = 8.4 Hz, 1.3 H), 6.49 (d,  $J$  = 16.0 Hz, 1 H, *E* isomer), 6.63–6.68 (m, 2H), 6.76 (d,  $J$  = 8.4 Hz, 2 H), 6.82 (d,  $J$  = 8.4 Hz, 2 H), 7.01–7.22 (m, 13.7 H), 7.29 (d,  $J$  = 16.4 Hz, 1 H, *E* isomer), 7.44–7.49 (m, 2 H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ) 13.50, 13.55, 27.32, 27.48, 28.99, 29.14, 114.46, 115.16, 125.28, 126.24, 126.30, 126.39, 126.68, 127.43, 127.91, 127.96, 128.17, 128.21, 129.02, 129.60, 130.18, 130.83, 131.41, 131.55, 132.10, 132.62, 135.01, 135.34, 137.46, 137.59, 141.99, 142.00, 142.30, 143.36, 143.51, 143.69, 146.15, 146.46, 153.81, 154.69, 198.78 ppm; HRMS (EI) exact mass calculated for  $\text{C}_{26}\text{H}_{24}\text{O}_2$ : 368.1776, found: 368.1774.

#### 5.4.7. Methyl(2*E*)-3-[4-[(1*E*)-2-phenyl-1-(4-*tert*-butoxyphenyl)-1-butenyl]phenyl]-2-propenoate (**13**)

A solution of potassium bis(trimethylsilyl)amide (0.5 M in toluene) (288  $\mu\text{l}$ , 0.14 mmol) was added to a stirring 0°C solution of trimethyl phosphonoacetate (23  $\mu\text{l}$ , 0.12 mmol) in tetrahydrofuran (0.8 ml). After stirring for 15 min at 0°C, the solution was cooled to –78°C, and a solution of a 2-phenyl-1-(4-*tert*-butoxyphenyl)-1-(4-formylphenyl)-but-1-ene (**12**) isomer mixture (46.2 mg, 0.10 mmol) in tetrahydrofuran (0.4 ml) was added dropwise. The reaction was stirred at –78°C for 5 min, then allowed to warm to room temperature and stirred 16 h overnight. The solution was poured into water, extracted with diethyl ether, washed with saturated sodium bicarbonate, dried over anhydrous magne-

sium sulfate, and the solvent removed under reduced pressure. The crude oil was purified by preparative thin layer chromatography (15% ethyl acetate–hexanes; eluted off silica with ethyl acetate) to give **13** as a yellow oil (50.8 mg, 0.12 mmol) in 96% yield, determined by  $^1\text{H}$  NMR to be a 2:1 mixture of *E*:*Z* isomers (at the triphenylethylene center):  $R_f$  0.48 (20% ethyl acetate–hexanes);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.93 (t,  $J$  = 7.6 Hz, 4.5 H), 1.24 (s, 4.5 H, *Z* isomer), 1.37 (s, 9 H, *E* isomer), 2.49 (q,  $J$  = 7.6 Hz, 3 H), 3.75 (s, 3 H), 3.81 (s, 1.5 H), 6.28 (d,  $J$  = 16.0 Hz, 1 H, *E* isomer), 6.45 (d,  $J$  = 16.0 Hz, 0.5 H, *Z* isomer), 6.63 (d,  $J$  = 8.8 Hz, 1 H), 6.73 (d,  $J$  = 8.4 Hz, 1 H), 6.88 (d,  $J$  = 8.4 Hz, 2 H), 6.97 (d,  $J$  = 8.4 Hz, 2 H), 7.06–7.17 (m, 11.5 H), 7.28 (d,  $J$  = 8.0 Hz, 1 H), 7.51 (d,  $J$  = 7.6 Hz, 1 H), 7.53 (d,  $J$  = 16.0 Hz, 1 H, *E* isomer), 7.71 (d,  $J$  = 16.0 Hz, 0.5 H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ) 13.47, 13.55, 28.74, 28.79, 28.89, 29.12, 51.54, 51.65, 78.21, 78.41, 116.83, 117.31, 123.08, 123.61, 126.16, 126.35, 127.18, 127.29, 127.73, 127.93, 128.00, 129.47, 129.57, 129.99, 130.05, 131.19, 131.29, 131.59, 132.64, 137.58, 137.74, 137.76, 141.90, 141.98, 142.57, 143.21, 144.62, 144.69, 145.64, 145.87, 153.40, 154.30, 167.45, 167.50 ppm; HRMS (EI) exact mass calculated for  $\text{C}_{30}\text{H}_{32}\text{O}_3$ : 440.2351, found: 440.2350.

#### 5.4.8. (2*E*)-3-[4-[(1*E*)-2-Phenyl-1-(4-*tert*-butoxyphenyl)-1-butenyl]phenyl]-2-propenoic acid (**16**)

To a 1:2 methanol:tetrahydrofuran (8 ml) solution of a methyl(2*E*)-3-[4-[2-phenyl-1-(4-*tert*-butoxyphenyl)-1-butenyl]phenyl]-2-propenoate (**13**) isomer mixture (50.8 mg, 0.12 mmol) at 25°C, was added dropwise a 0.2 M solution of aqueous potassium hydroxide (5.76 ml, 1.19 mmol). The reaction was stirred 24 h overnight at 25°C, then poured into 1 N hydrochloric acid. After stirring for 10 min, the solution was extracted with methylene chloride, dried over anhydrous magnesium sulfate, and the solvent removed under reduced pressure. The crude oil was purified by preparative thin layer chromatography (7.5% methanol–chloroform; eluted off silica using ethyl acetate) to give **16** as a yellow oil (29.5 mg, 0.069 mmol) in 58% yield, determined by  $^1\text{H}$  NMR to be a 2:1 mixture of *E*:*Z* isomers (at the triphenylethylene center):  $R_f$  0.31 (5% methanol–chloroform);  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  0.85 (t,  $J$  = 7.6 Hz, 4.5 H), 1.14 (s, 4.5 H, *Z* isomer), 1.28 (s, 9 H, *E* isomer), 2.42 (q,  $J$  = 7.6 Hz, 3 H), 6.23 (d,  $J$  = 16.0 Hz, 1 H, *E* isomer), 6.31 (d,  $J$  = 16.0 Hz, 0.5 H, *Z* isomer), 6.55 (d,  $J$  = 8.4 Hz, 1 H), 6.69 (d,  $J$  = 8.8 Hz, 1 H), 6.82 (d,  $J$  = 8.4 Hz, 2 H), 6.92 (d,  $J$  = 8.8 Hz, 2 H), 7.01–7.10 (m, 9.5 H), 7.14 (d,  $J$  = 8.4 Hz, 2 H), 7.21 (d,  $J$  = 8.0 Hz, 1 H), 7.42 (d,  $J$  = 15.6 Hz, 1 H, *E* isomer), 7.52 (d,  $J$  = 8.0 Hz, 1 H), 7.62 (d,  $J$  = 16.0 Hz, 0.5 H, *Z* isomer);  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ ) 13.77, 13.82, 29.10, 29.24, 29.76, 29.99, 79.50, 79.65, 118.64, 119.15, 119.62, 124.20, 124.87, 127.36, 127.55, 128.33, 128.88, 129.05, 129.16, 130.81, 131.13, 132.43, 133.23, 133.49, 134.32, 138.01, 139.30, 139.34, 139.45, 140.50, 142.92, 143.29, 143.33, 143.98, 144.55, 146.03, 146.34, 147.02, 147.09, 154.68, 155.62, 170.40 ppm; HRMS (EI) exact mass calculated for  $\text{C}_{29}\text{H}_{30}\text{O}_3$ : 426.2195, found: 426.2195.

#### 5.4.9. (2*E*)-3-[4-[(1*E*)-2-Phenyl-1-(4-hydroxyphenyl)-1-butenyl]phenyl]-2-propenoic acid (GW-7604) (**6**)

To a solution of a (2*E*)-3-[4-[2-phenyl-1-(4-*tert*-butoxyphenyl)-

1-butenyl]phenyl]-2-propenoic acid (**16**) isomer mixture (29.5 mg, 0.069 mmol) at 0°C in dichloromethane (1 ml) was added 2,2,2-trifluoroethanol (0.5 ml), followed by trifluoroacetic acid (1 ml). The reaction was stirred for 10 min at 0°C, then allowed to warm to room temperature and stirred for 1.5 h. The solution was then poured into 1 N hydrochloric acid, extracted with dichloromethane, dried over anhydrous magnesium sulfate, and solvent removed under reduced pressure. The crude oil was purified by preparative thin layer chromatography (7.5% methanol–chloroform), where upon elution with ethyl acetate off the silica resulted in formation of a white precipitate. Solvent was removed under reduced pressure, and the residue redissolved in methanol. Separation of the precipitate and solvent by centrifugation, allowed separation of the pure triphenylethylene *Z* isomer as a white solid (2.3 mg, 0.0062 mmol, 9% yield), and a 2:1 mixture of the *E*:*Z* isomers of **6** as a yellow oil (5.2 mg, 0.014 mmol, 20% yield):  $R_f$  0.31 (5% methanol–chloroform);  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ ) of *E*:*Z* mixture  $\delta$  0.85 (t,  $J$  = 7.6 Hz, 4.5 H), 2.44 (q,  $J$  = 7.6 Hz, 3 H), 6.23 (d,  $J$  = 16.0 Hz, 1 H, *E* isomer), 6.34 (d,  $J$  = 8.8 Hz, 1 H), 6.41 (d,  $J$  = 16.0 Hz, 0.5 H), 6.58 (d,  $J$  = 8.4 Hz, 1 H), 6.71 (d,  $J$  = 8.8 Hz, 2 H), 6.82 (d,  $J$  = 8.0 Hz, 2 H), 6.96 (d,  $J$  = 8.4 Hz, 2 H), 7.00–7.10 (m, 7.5 H), 7.14 (d,  $J$  = 8.4 Hz, 2 H), 7.19 (d,  $J$  = 8.4 Hz, 1 H), 7.43 (d,  $J$  = 16.0 Hz, 1 H, *E* isomer), 7.51 (d,  $J$  = 8.0 Hz, 1 H), 7.62 (d,  $J$  = 16.0 Hz, 0.5 H, *Z* isomer);  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ ) of *E* isomer 13.80, 30.01, 116.04, 118.56, 127.42, 128.23, 129.02, 130.85, 131.67, 132.49, 133.12, 135.53, 139.64, 143.57, 144.09, 146.06, 147.41, 157.58, 170.52 ppm;  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ ) of *Z* isomer  $\delta$  0.86 (t,  $J$  = 7.6 Hz, 3 H), 2.40 (q,  $J$  = 7.6 Hz, 2 H), 6.35 (d,  $J$  = 8.8 Hz, 2 H), 6.41 (d,  $J$  = 16.0 Hz, 1 H), 6.59 (d,  $J$  = 8.8 Hz, 2 H), 7.03–7.12 (m, 5 H), 7.20 (d,  $J$  = 8.0 Hz, 2 H), 7.52 (d,  $J$  = 8.4 Hz, 2 H), 7.61 (d,  $J$  = 16.0 Hz, 1 H); HRMS (EI) exact mass calculated for  $\text{C}_{25}\text{H}_{22}\text{O}_3$ : 370.1569, found: 370.1562.

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